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Clinical and molecular features of methicillin-resistant, coagulase-negative staphylococci of pets and horses

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Objectives: To determine the antibiotic resistance and fingerprint profiles of methicillin-resistant coagulase-negative staphylococci (MRCoNS) from animal infections among different practices and examine the history of antibiotic treatment.

Methods: Isolates were identified by mass spectrometry and tested for antimicrobial resistance by broth dilution, microarrays and sequence analysis of the topoisomerases. Diversity was assessed by PFGE, *icaA* PCR and staphylococcal cassette chromosome *mec* (SCC*mec*), arginine catabolic mobile element (ACME) and multilocus sequence typing. Clinical records were examined retrospectively.

Results: MRCoNS were identified as *Staphylococcus epidermidis* ($n=20$), *Staphylococcus haemolyticus* ($n=17$), *Staphylococcus hominis* ($n=3$), *Staphylococcus capitis* ($n=1$), *Staphylococcus cohnii* ($n=1$) and *Staphylococcus warneri* ($n=1$). PFGE identified one clonal lineage in *S. hominis* isolates and several in *S. haemolyticus* and *S. epidermidis*. Fourteen sequence types were identified in *S. epidermidis*, with sequence type 2 (ST2) and ST5 being predominant. Ten isolates contained SCC*mec* IV, seven contained SCC*mec* V and the others were non-typeable. ACMEs were detected in 11 *S. epidermidis* isolates. One *S. hominis* and 10 *S. epidermidis* isolates were *icaA* positive. In addition to *mecA*-mediated β-lactam resistance, the most frequent resistance was to gentamicin/kanamycin [*aac(6′)-Ie–aph(2′)-Ia*, *aph(3′)-III*] ($n=34$), macrolides/lincosamides [*erm*(C), *erm*(A), *msr*, *lnu*(A)] ($n=31$), tetracycline [*tet*(K)] ($n=22$), streptomycin [*str*, *ant(6)-Ia*] ($n=20$), trimethoprim [*dfr*(A), *dfr*(G)] ($n=17$), sulfamethoxazole ($n=34$) and fluoroquinolones [amino acid substitutions in GyrA and GrlA] ($n=30$). Clinical data suggest selection through multiple antibiotic courses and emphasize the importance of accurate diagnosis and antibiograms.

Conclusions: MRCoNS from animal infection sites are genetically heterogeneous multidrug-resistant strains that represent a new challenge in the prevention and therapy of infections in veterinary clinics.

Keywords: animals, infections, antimicrobial resistance, genotyping, *mecA*, CoNS, ACME, MLST

Introduction

Coagulase-negative staphylococci (CoNS) are frequently found on the skin and mucous membranes of humans and animals.[1] They are opportunistic pathogens and are one of the most frequent causes of nosocomial infections in humans, which are mainly associated with immune-compromised patients or with the implantation of medical devices.[2–6] *Staphylococcus epidermidis* is the most frequent CoNS causing infection in humans, and 70% of the *S. epidermidis* strains circulating in the human hospital environment have been estimated to be resistant to methicillin and most of them display additional resistance to other classes of antibiotics.[7] The acquisition of methicillin resistance in staphylococci results from the recombinase-mediated insertion of the staphylococcal chromosomal cassette *mec* (SCC*mec*), the mobile genetic element that carries *mecA*.[8,9] The *mecA* gene encodes the binding protein PBP2a, which mediates resistance to all β-lactam antibiotics in staphylococci.[10] Other methicillin-resistant CoNS (MRCoNS), such as *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus capitis*, *Staphylococcus sciuri*, *Staphylococcus warneri* and *Staphylococcus saprophyticus*, have also been described as causes of clinical human infections.[11–13] In some *S. epidermidis* strains, the SCC*mec* elements have been found to be associated with the arginine catabolic mobile element (ACME), enhancing fitness and the ability to colonize the host.[14–16] These characteristics

associated with the ability to produce a biofilm are important factors for establishing CoNS, especially *S. epidermidis*, as nosocomial pathogens.[2,17,18]

In veterinary medicine, many different classes of antibiotics are used for the treatment of infections. The use of such antibiotics has likely selected for an antibiotic-resistant commensal flora, as healthy pets and horses have been found to be colonized with MRCoNS.[4,19–22] However, very few reports describe cases of infections caused by MRCoNS in these animals,[23–25] although several studies have reported infections with methicillin-susceptible CoNS.[26–30] In the past 4 years, MRCoNS have been isolated from the infection sites of pets and horses in Switzerland. The genetic backgrounds of these multidrug-resistant clinical isolates and their clonal relationships remained to be elucidated. This study provides the first substantial molecular characterization of MRCoNS associated with infections in pets and horses and determines whether specific clones are becoming established in veterinary settings. The history of antibiotic usage as well as the treatment and outcome of the infections are also provided to support the hypothesis that several courses of different antibiotics may have selected for multidrug-resistant CoNS. This study may also serve as a basis for future epidemiological and prevalence studies of MRCoNS circulating in veterinary clinics and other animal environments.

Materials and methods

Sample collection, isolation and identification

Samples were taken by veterinarians from different infection sites of pets and horses that did not respond to antibiotic therapy and sent for identification of the causative agents and antibiograms to the Centre for Zoonoses, Bacterial Animal Diseases and Antibiotic Resistance (ZOBA) of the Institute of Veterinary Bacteriology, University of Bern, Bern, Switzerland, the IDEXX Diavet Laboratory, Bäch, Switzerland, or the Laboratory Laupeneck AG, Bern, Switzerland. Isolates of MRCoNS that appeared to be the primary pathogens [either as single pathogenic agent ($n=40$) or together with a second pathogen ($n=3$)] were kept at −80°C and made available for this study. A total of 43 isolates were collected between 2005 and 2011 (see Tables 1 and 2). They were routinely cultivated on Tryptone soy agar containing 5% sheep blood (TSA-SB) (Oxoid Ltd, Basingstoke, England) and incubated aerobically for 18 h at 37°C. Species identification was determined phenotypically using Vitek2 (bioMérieux, Marcy l'Étoile, France) and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDITOF-MS) (Microflex LT, Bruker Daltonik, Bremen, Germany).

Genotyping

PFGE was performed on DNA digested with SmaI as described previously.[23] PFGE was run on a CHEF DRIII apparatus (Bio-Rad, Hercules, CA, USA) for 21 h at 6 V/cm and with pulse time ramping from 5 to 40 s at 12°C. The PFGE profiles were defined on the basis of DNA banding patterns in compliance with the criteria of Tenover *et al.*[31] for bacterial strain typing using the BioNumerics software (version 6.6, Applied Maths, Saint-Martens-Latem, Belgium).

SCC*mec* typing was determined by multiplex PCR.[32] SCC*mec* types were defined by the combination of the type of *ccr* complex and the class of *mec* complex: SCC*mec* type I (*mec* complex B, *ccrAB1*), SCC*mec* type II (*mec* complex A, *ccrAB2*), SCC*mec* type III (*mec* complex A, *ccrAB3*), SCC*mec* type IV (*mec* complex B, *ccrAB2*) and SCC*mec* type V (*mec* complex C, *ccrC*). SCC*mec* was classified as non-typeable when the *ccr* complex, the *mec* complex or both could not be amplified by PCR.

The presence and type of ACMEs were determined by PCR using the primer pairs AIPS.27-AIPS.28 (*arcA*) and AIPS.45-AIPS.46 (*opp3A*) as described previously.[33] ACMEs were classified into three allotypes: ACME type I containing both the *arc* and *opp-3* gene clusters, ACME type II containing *arc* but not *opp-3* and ACME type 3 containing *opp-3* but not *arc*.[14] The presence of the biofilm-formation operon *ica* was determined by amplification of the *icaA* gene by PCR.[34] *S. epidermidis* samples were characterized by multilocus sequence typing (MLST).[35] PCR amplifications were routinely performed using FIREPol DNA polymerase (Solis BioDyne, Tartu, Estonia), except for SCC*mec* typing, which was performed with the Expand Long Template PCR System (Roche Applied Science, Rotkreuz, Switzerland).

Determination of the antibiotic resistance profile

MICs were determined in Mueller–Hinton broth by use of custom Sensititre NLEUST plates (Trek Diagnostics Systems, East Grinstead, UK; MCS diagnostics BV, JL Swalmen, the Netherlands). The MIC breakpoints determining resistance were those recommended for staphylococci by EUCAST (www.eucast.org), except for streptomycin and kanamycin, for which breakpoints came from the French Society for Microbiology (www.sfm-microbiologie.org), and sulfamethoxazole, for which they came from the CLSI.[36] No breakpoint was available for tiamulin and resistance was attributed after the detection of a tiamulin resistance gene. The antimicrobial agents tested and breakpoints used consisted of chloramphenicol (>8 mg/L), ciprofloxacin (>1 mg/L), clindamycin (>0.5 mg/L), erythromycin (>2 mg/L), fusidic acid (>1 mg/L), gentamicin (>1 mg/L), kanamycin (>16 mg/L), linezolid (>4 mg/L), mupirocin (>256 mg/L), oxacillin (>0.25 mg/L), penicillin (>0.125 mg/L), quinupristin/dalfopristin (>4 mg/L), rifampicin (>0.5 mg/L), streptomycin (>16 mg/L), tetracycline (>2 mg/L), tiamulin (resistance breakpoint not available), trimethoprim (>4 mg/L), sulfamethoxazole (>256 mg/L) and vancomycin (>2 mg/L). Antibiotic resistance genes were detected using a custom-made microarray (AMR + ve-2 array tubes, Alere Technologies GmbH, Jena, Germany).[37] The microarray results were analysed using the IconoClust program (Alere) and the signals obtained were interpreted visually. The acquired trimethoprim resistance dihydrofolate reductase gene *dfr*(A) in *S. epidermidis* was distinguished from the chromosomal *dfr*(A) (=*folA*) by PCR using one primer specific to *dfr*(A) and one primer specific to IS*431*, which is only situated downstream of the acquirable *dfr*(A) gene and not downstream of the chromosomal *dfr*(A) of *S. epidermidis* (Table S1, available as Supplementary data at *JAC* Online).

Mutations in the fluoroquinolone resistance coding region of the topoisomerase II (GyrA and GyrB) and IV (GrlA and GrlB) genes were determined by sequence analysis of PCR products obtained using the primers listed in Table S1 (available as Supplementary data at *JAC* Online). Mutations were detected by comparison of the amino acid sequences of GyrA, GyrB, GrlA and GrlB of fluoroquinolone-susceptible *S. epidermidis* ATCC12228 (GenBank accession number AE015929), *S. haemolyticus* JCSC1435 (GenBank accession number NC_007168) and *S. hominis* SN-013-2010-6-23-5 (GenBank accession numbers HE820118 and HE856265).

Clinical data and statistical analysis

Clinical records of animals that developed an infection containing MRCoNS were examined retrospectively when available. The following data were recorded: underlying diseases, history of antibiotic treatments, specific antibiotic treatment of the infection and outcome (see Table 3). PASS 2008 software (NCCS, Kaysville, UT, USA) was used to conduct a Fisher's exact test (two-tailed) with the level of significance set at a P value <0.05.

Table 1. Origin and resistance profile of methicillin-resistant *S. epidermidis* isolated from infection sites of animals

					Antibiotic resistance properties and resistance breakpoints (mg/L)																
					OXA	PEN	GEN/KAN	KAN	STR	STH	ERY	CLI	TMP	TET	CHL	TIA	MUP	FUS	SMX	CIP	
																				>1	
Isolate ($n=20$)	Year of isolation	Animal	Infection	Sequence type	>0.25	>0.125	>1/>16	>16	>16	ND	>2	>0.5	>4	>2	>8	NA	>256	>1	>256	GyrA	GrlA
CSNO38	2005	horse	dermis	ST446	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*	*aph(3′)-III*	*ant(6)-Ia, str*	*sat4*	*erm*(C)	*erm*(C)	*dfr*(A)	*tet*(K)					R		
KM794-06	2006	horse	abscess	ST89	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*							*tet*(K)							
KM1527-07	2007	cat	joint	ST22	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*						*dfr*(A)							S84Y	S80F
KM827-09	2009	cat	respiratory tract	ST59	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*	*aph(3′)-III*	*str*	*sat4*				*tet*(K)					R		
KM505-09	2009	cat	urinary tract	ST22	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*				*erm*(C)	*erm*(C)	*dfr*(A)	*tet*(K)	cat_{pC223}			R		S84Y	D84Y
KM1077-09	2009	dog	abscess	ST2	*mecA*	*blaZ*							*dfr*(A)				*mupR*	R	R	S84Y	S80Y/D84Y
KM92-09	2009	dog	abscess	ST2	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*						*dfr*(A)						R		
KM825-09	2009	horse	abscess	ST451	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*											R		S84F	D84Y
IMD1265-11	2011	horse	dermis	ST69	*mecA*		*aac(6′)-Ie – aph(2′)-Ia*				*erm*(C)	*erm*(C)		*tet*(K)			*mupR*				
IMD1274-11	2011	cat	dermis	ST5	*mecA*	*blaZ*					*erm*(C)	*erm*(C)		*tet*(K)						S84F	S80Y
IMD1763-11	2011	cat	urinary tract	ST81	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*							*tet*(K)							
IMD1270-11	2011	cat	abscess	ST2	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*	*aph(3′)-III*	*ant(6)-Ia*	*sat4*	*erm*(C)	*erm*(C)	*dfr*(A)	*tet*(K)		*vga*(A)			R	S84F	S80F/D84Y
IMD1269-11	2011	cat	urinary tract	ST445	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*				*erm*(C), *msr*, *mph*(C)	*erm*(C)						R	R	S84F	S80Y
IMD1528-11	2011	cat	dermis	ST448	*mecA*	*blaZ*		*aph(3′)-III*			*erm*(C), *mph*(C)	*erm*(C)	*dfr*(G)								
IMD1776-11	2011	cat	eye	ST286	*mecA*	*blaZ*	R	R			*erm*(C)	*erm*(C)	*dfr*(G)								
IMD1766-11	2011	dog	ear	ST5	*mecA*	*blaZ*					*erm*(C)	*erm*(C)						R		S84F	S80F/D84Y
IMD1778-11	2011	dog	respiratory tract	ST5	*mecA*	*blaZ*												R		S84F	S80Y
KM1385-1972	2011	dog	joint	ST450	*mecA*	*blaZ*												R	R		
IMD1764-11	2011	dog	respiratory tract	ST449	*mecA*	*blaZ*					*erm*(C), *mph*(C)	*erm*(C)						R			
IMD1765-11	2011	dog	respiratory tract	ST2	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*		*str*	*sat4*	*erm*(C)	*erm*(C)	*dfr*(A)		cat_{pC221}			R	R	S84F	S80F/D84Y

CHL, chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; FUS, fusidic acid; GEN, gentamicin; KAN, kanamycin; MUP, mupirocin; OXA, oxacillin; PEN, penicillin; STR, streptomycin; STH, streptothricin; TET, tetracycline; TIA, tiamulin; TMP, trimethoprim, SMX, sulfamethoxazole; ND, not defined, susceptibility to streptothricin was not measured, only the gene was detected; NA, no resistance breakpoint available for tiamulin [resistance to tiamulin was attributed in the presence of the *vga*(A) gene (MIC >4 mg/L)]; R, resistant phenotype, no resistance genes were determined; blank spaces indicate either no resistance or no mutations.

The MIC breakpoints (in mg/L) that determine resistance were those recommended by EUCAST for staphylococci (www.eucast.org). Resistance breakpoints for streptomycin and kanamycin were those recommended by the French Society for Microbiology (www.sfm-microbiologie.org) and the resistance breakpoint for sulfamethoxazole was that recommended by the CLSI.[36]

Antibiotic resistance genes and their functions are indicated as follows: *mecA*, methicillin-resistance gene encoding PBP2a for resistance to all β-lactam antibiotics; *blaZ*, β-lactamase gene; *aac(6′)-Ie–aph(2′)-Ia*, aminoglycoside acetyltransferase and phosphotransferase tandem genes; *aph(3′)-III*, kanamycin phosphotransferase; *ant(6)-Ia*, streptomycin adenylnucleotidyltransferase gene; *str*, streptomycin adenyltransferase gene; *sat4*, strepthothricin acetyltransferase gene; *erm*(C), macrolide, lincosamide and streptogramin B 23S rRNA methylase gene; *msr*, macrolide and streptogramin ATP binding transporter gene; *mph*(C), macrolide phosphotransferase gene; *mupR*, isoleucyl-tRNA synthetase gene; *dfr*(A), *dfr*(G), trimethoprim resistance dihydrofolate reductase gene; *tet*(K), tetracycline efflux resistance gene; cat_{pC221}, cat_{pC223}, chloramphenicol acetyltransferase gene; *vga*(A), pleuromutilin and streptogramin A ATP binding transporter gene.

Table 2. Origin and resistance profile of methicillin-resistant *S. haemolyticus*, *S. hominis*, *S. capitis*, *S. cohnii* and *S. warneri* isolated from infection sites of animals

				Antibiotic resistance properties and resistance breakpoints (mg/L)															
				OXA	PEN	GEN/KAN	KAN	STR	STH	ERY	CLI	TMP	TET	CHL	TIA	FUS	SMX	CIP	
																		>1	
Strain/isolate	Year of isolation	Animal	Infection	>0.25	>0.125	>1/>16	>16	>16	ND	>2	>0.5	>4	>2	>8	NA	>1	>256	GyrA	GrlA
S. haemolyticus (*n*=17)																			
KM827-07	2007	horse	abscess	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*		*ant(6)-Ia*		*erm*(C), *msr*, *mph*(C)	*erm*(C), *lnu*(A)	*dfr*(G)	*tet*(K)	cat_{pC221}	*vga*(A)		R	S84L	
KM1758-08	2008	cat	urinary tract	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*	*aph(3′)-III*	*ant(6)-Ia*	*sat4*	*msr*, *mph*(C)	R	*dfr*(G)			*vga*(A)		R	S84L	
KM1632-08	2008	cat	urinary tract	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*	*aph(3′)-III*	*ant(6)-Ia*	*sat4*	*msr*, *mph*(C)							R	S84L	
KM785-09	2009	dog	abscess	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*		*ant(6)-Ia*		*msr*, *mph*(C)		*dfr*(G)	*tet*(K)	cat_{pC221}			R	S84L	
KM1183-09	2009	horse	dermis	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*	*aph(3′)-III*	*ant(6)-Ia*		*erm*(C), *msr*, *mph*(C)	*erm*(C)		*tet*(K)	cat_{pC223}	DS		R	S84L	
KM1230-09	2009	horse	respiratory tract	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*	*aph(3′)-III*	*ant(6)-Ia*	*sat4*			*dfr*(G)	*tet*(K)				R	S84L	
IMD1272-11	2011	cat	urinary tract	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*		*str*		*msr*	*lnu*(A)		*tet*(K)	cat_{pC221}			R	S84L	
IMD1277-11	2011	cat	dermis	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*	*aph(3′)-III*	*ant(6)-Ia*	*sat4*	*msr*					*vga*(A)	R	R	S84F	
IMD1517-11	2011	cat	urinary tract	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*	*aph(3′)-III*	*ant(6)-Ia*	*sat4*			*dfr*(G)	*tet*(K)				R	S84F	
IMD1519-11	2011	cat	dermis	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*	*aph(3′)-III*		*sat4*	*erm*(C)	*erm*(C)		*tet*(K)					S84L	
IMD1521-11	2011	cat	dermis	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*	*aph(3′)-III*	*ant(6)-Ia*	*sat4*	*msr*		*dfr*(G)					R	S84L	D84Y
IMD1266-11	2011	dog	dermis	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*		*ant(6)-Ia*		*erm*(C)	*erm*(C), *lnu*(A)						R	S84L	
IMD1397-11	2011	dog	ear	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*		*ant(6)-Ia*		*msr*	*lnu*(A)		*tet*(K)				R	S84F	
IMD1532-11	2011	dog	dermis	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*	*aph(3′)-III*		*sat4*	*msr*		*dfr*(G)	*tet*(K)				R	S84L	
IMD1761-11	2011	dog	abscess	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*	*aph(3′)-III*	*ant(6)-Ia*	*sat4*	*erm*(C)	*erm*(C)						R	S84L	
IMD1768-11	2011	dog	abscess	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*	*aph(3′)-III*	*str*	*sat4*	*erm*(C)	*erm*(C)		*tet*(K)	cat_{pC223}			R	S84L	
IMD1775-11	2011	dog	urinary tract	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*	*aph(3′)-III*	*ant(6)-Ia*	*sat4*	*erm*(C), *msr*	*erm*(C)	*dfr*(G)	*tet*(K)			R	R	S84L	
S. hominis (*n*=3)																			
IMD1515-11	2011	dog	abscess	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*	*aph(3′)-III*			*erm*(C)	*erm*(C)		*tet*(K)				R	S84F	G84Y
IMD1516-11	2011	dog	joint	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*				*erm*(C)	*erm*(C)		*tet*(K)				R	S84F	G84Y
IMD1762-11	2011	dog	ear	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*				*erm*(C)	*erm*(C)		*tet*(K)				R	S84F	G84Y
S. capitis (*n*=1)																			
KM1385-1970	2011	dog	joint	*mecA*												R			
S. cohnii (*n*=1)																			
IMD1771-11	2011	dog	urinary tract	*mecA*						*erm*(A)	*erm*(A)				DS	R			
S. warneri (*n*=1)																			
IMD1530-11	2011	dog	ear	*mecA*	*blaZ*	*aac(6′)-Ie – aph(2′)-Ia*		*str*		*erm*(C)	*erm*(C)			cat_{pC221}					

CHL, chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; FUS, fusidic acid; GEN, gentamicin; KAN, kanamycin; OXA, oxacillin; PEN, penicillin; STR, streptomycin; STH, streptothricin; TET, tetracycline; TIA, tiamulin; TMP, trimethoprim; SMX, sulfamethoxazole; ND, not defined, susceptibility to streptothricin was not measured, only the gene was detected; NA, no resistance breakpoint available for tiamulin [resistance to tiamulin was attributed in the presence of the *vga*(A) gene (MIC >4 mg/L)]; DS, decreased susceptibility to tiamulin with MIC >4 mg/L); R, resistant phenotype, no resistance genes were determined; blank spaces indicate either no resistance or no mutations.

The MIC breakpoints (in mg/L) that determine resistance were those recommended by EUCAST for staphylococci (www.eucast.org). Resistance breakpoints for streptomycin and kanamycin were those recommended by the French Society for Microbiology (www.sfm-microbiologie.org) and the resistance breakpoint for sulfamethoxazole was that recommended by the CLSI.[36]

Antibiotic resistance genes and their functions are indicated as follows: *mecA*, methicillin-resistance gene encoding PBP2a for resistance to all β-lactam antibiotics; *blaZ*, β-lactamase gene; *aac(6′)-Ie–aph(2′)-Ia*, aminoglycoside acetyltransferase and phosphotransferase tandem genes; *aph(3′)-III*, kanamycin phosphotransferase; *ant(6)-Ia*, streptomycin adenylnucleotidyltransferase gene; *str*, streptomycin adenyltransferase gene; *sat4*, strepthotricin acetyltransferase gene; *erm*(C), macrolide, lincosamide and streptogramin B 23S rRNA methylase gene; *msr*, macrolide and streptogramin ATP binding transporter gene; *mph*(C), macrolide phosphotransferase gene; *dfr*(A), *dfr*(G), trimethoprim resistance dihydrofolate reductase gene; *lnu*(A), lincosamide nucleotidyltransferase gene; *tet*(K), tetracycline efflux resistance gene; cat_{pC221}, cat_{pC223}, chloramphenicol acetyl transferase gene; *vga*(A), pleuromutilin and streptogramin A ATP binding transporter gene.

Table 3. Clinical data, therapy and outcome of treatment of infections associated with MRCoNS in animals (dogs, cats and horses)

Animals ($n=27$) and CoNS	Strains	Type of infection	History of antibiotic treatment before identification of the *Staphylococcus* (no. of courses)	Resistance profile of isolated *Staphylococcus* from infection side	Antibiotics used for treatment of the *Staphylococcus* infection	Incompatibility with resistance mechanism	Outcome
Dogs ($n=11$)							
S. haemolyticus	KM 785-09	abscess (granuloma)	amox-clav (1), clindamycin (2)	PEN, OXA, KAN, GEN, STR, ERY, TET, TMP, CHL	clindamycin	no	recovery
S. capitis *S. epidermidis*	KM1385-1970 KM1385-1972	joint (surgery)	amox-clav (1), clindamycin (2)	PEN, OXA	amox-clav, clindamycin	no	unknown
S. haemolyticus	IMD1266-11	eye (chronic conjunctivitis)	cefovecin (1), neomycin/polymyxin B (2)	PEN, OXA, STR, ERY, CLI, CIP	tetracycline	no	relapse if treatment with tetracycline stops
S. epidermidis	IMD1765-11	respiratory tract (chronic cough)	amox-clav (1), amox-clav (2), amox-clav (3),	PEN, OXA, KAN, GEN, STR, ERY, TMP, CLI, CHL	marbofloxacin, tetracycline	no	recovery after a 4 week therapy
S. hominis	IMD1762-11	ear (otitis externa)	no antibiotics	PEN, OXA, KAN, GEN, ERY, CLI, TET, CIP	framycetin	yes [*aac(6′)-Ie – aph(2′)-Ia*]	recovery
S. warneri	IMD1530-11	ear (chronic otitis externa, relapse)	polymyxin B (1), marbofloxacin (2), cefalexin (3), marbofloxacin (4)	PEN, OXA, KAN, GEN, STR, ERY, CLI, CHL	marbofloxacin	no	relapse
S. epidermidis	IMD1766-11	ear (chronic otitis externa)	metronidazole (1), fusidic acid/framycetin (2), polymyxin B (3), amox-clav (4), enrofloxacin (5)	PEN, OXA, ERY, CLI, CIP	amox-clav	yes *(mecA)*	recovery
S. haemolyticus	IMD1775-11	urinary tract (preputial catarrh)	amox-clav (1), polymyxin B (2)	PEN, OXA, KAN, GEN, STR, ERY, TMP, CLI, TET, CIP	amox-clav	yes *(mecA)*	recovery
S. haemolyticus	IMD1768-11	abscess	amox-clav/tetracycline (1), amox-clav (2), amox-clav (3), tetracycline (4), amox-clav (5)	PEN, OXA, KAN, GEN, STR, ERY, TMP, CLI, CHL, TET, CIP	cefalexin	yes *(mecA)*	relapse
S. haemolyticus	IMD1761-11	abscess	amox-clav (1), amox-clav/chloramphenicol (2), amox-clav/chloramphenicol (3), aminoglycoside/polymyxin B (4)	PEN, OXA, KAN, GEN, STR, ERY, CLI, CIP	chloramphenicol	no	recovery
S. hominis	IMD1515-11	dermis (ulcer)	amox-clav (1), cefalexin (2)	PEN, OXA, KAN, GEN, STR, ERY, CLI, TET, CIP	no treatment	NA	euthanasia
Cats ($n=11$)							
S. haemolyticus	KM1758-08	urinary tract	marbofloxacin (1), amox-clav (2), rifampicin (3)	PEN, OXA, KAN, GEN, STR, ERY, TMP, CIP, TIA	rifampicin	no	recovery
S. haemolyticus	KM1632-08	urinary tract	amox-clav (1), trimethoprim/sulphonamide (2)	PEN, OXA, KAN, GEN, STR, ERY	trimethoprim/sulphonamide	no	recovery

S. epidermidis	KM1527-07	abscess after surgery	marbofloxacin (1), amox-clav (2)	PEN, OXA, KAN, GEN, STR, ERY, TMP, TET, TIA	marbofloxacin, tetracycline	yes [*tet*(K)]	recovery after amputation of the lower extremity
S. epidermidis	KM505-09	urinary tract	enrofloxacin (1), amoxicillin (2), marbofloxacin (3)	PEN, OXA, KAN, GEN, TMP, CLI, TET, CHL, CIP	no treatment	NA	recovery
S. haemolyticus	IMD1272-11	urinary tract (urolithiasis, chronic cystitis)	amox-clav (1)	PEN, OXA, KAN, GEN, STR, ERY, CLI, TET, CHL, CIP	clindamycin	yes [*erm*(C)]	recovery
S. haemolyticus	IMD1517-11	urinary tract	unknown	PEN, OXA, KAN, GEN, STR, TMP, TET, CIP	no treatment (fast death)	NA	death
S. epidermidis	IMD1776-11	eye (corneal ulcer)	enrofloxacin (1), moxifloxacin (2), gentamicin (3)	PEN, OXA, KAN, GEN, STR, ERY, TMP, CLI, CHL, CIP	ofloxacin, tetracycline	no	recovery (together with cross-linking therapy)
S. epidermidis	IMD1763-11	urinary tract (urolithiasis, chronic cystitis)	cefovecin (1)	PEN, OXA, KAN, GEN, TET	marbofloxacin	no	relapse (cystitis with *Enterococcus*)
S. epidermidis	IMD1270-11	phlegmone (acneic skin)	cefovecin (1)	PEN, OXA, KAN, GEN, STR, ERY, TMP, TET, TIA; CIP	chloramphenicol	no	recovery
S. epidermidis	IMD1269-11	urinary tract (cystitis)	marbofloxacin (1), amox-clav (2)	PEN, OXA, KAN, GEN, ERY, CLI, CIP	amox-clav	yes (*mecA*)	recovery
S. epidermidis	IMD1274-11	eye (conjunctivitis)	amox-clav (1), enrofloxacin/amoxicillin (2), amoxicillin (3), amoxicillin (4), amoxicillin (5), amoxicillin (6), bacitracin/neomycin/ofloxacin (7), amoxicillin (8), ciprofloxacin (9), amoxicillin (10), cefovecin (11), ciprofloxacin/amoxicillin(12), amoxicillin (13), amoxicillin (14)	PEN, OXA, ERY, CLI, TET, CIP	neomycin/polymyxin B	no	relapse
Horses ($n=5$)							
S. epidermidis	KM794-06	abscess	penicillin	PEN, OXA, KAN, GEN, TET	unknown	NA	recovery
S. epidermidis	KM825-09	abscess (surgery)	cefquinome (1), penicillin/gentamicin (2)	PEN, OXA, KAN, GEN	no treatment	NA	euthanasia
S. haemolyticus	KM827-07	wound	penicillin/gentamicin (1), enrofloxacin (2), cefquinome (3)	PEN, OXA, KAN, GEN, STR, ERY, TMP, CLI, TET, CHL, TIA	unknown	NA	recovery
S. haemolyticus	KM1183-09	dermis	trimethoprim/sulphonamide (1), cefquinome (2), marbofloxacin/enrofloxacin (3)	PEN, OXA, KAN, GEN, STR, ERY, CLI, TET, CHL	gentamicin	yes [*aac(6′)-Ie – aph(2′)-Ia*]	relapse

Continued

Table 3. *Continued*

Animals ($n=27$) and CoNS	Strains	Type of infection	History of antibiotic treatment before identification of the *Staphylococcus* (no. of courses)	Resistance profile of isolated *Staphylococcus* from infection side	Antibiotics used for treatment of the *Staphylococcus* infection	Incompatibility with resistance mechanism	Outcome
S. haemolyticus	KM1230-09	respiratory tract (BAL)	unknown	PEN, OXA, KAN, GEN, STR, STH, TMP, TET, CIP	no treatment	NA	recovery

amox-clav, amoxicillin/clavulanic acid; CHL, chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; KAN, kanamycin; OXA, oxacillin; PEN, penicillin; STR, streptomycin; TET, tetracycline; TIA, tiamulin; TMP, trimethoprim; NA, not available; BAL, bronchoalvelar lavage.
mecA, methicillin-resistance gene encoding PBP2a for resistance to all β-lactam antibiotics (e.g. penicillin, amoxicillin, amoxicillin/clavulanic acid and cefalexin); *aac(6′)-Ie–aph(2′)-Ia*, aminoglycoside acetyltransferase and phosphotransferase tandem genes (gentamicin/kanamycin/neomycin); *erm*(C), macrolide, lincosamide and streptogramin B 23S rRNA methylase gene (clindamycin); *tet*(K), tetracycline efflux resistance gene (tetracycline).

Results

Identification of and genetic diversity among strains of MRCoNS

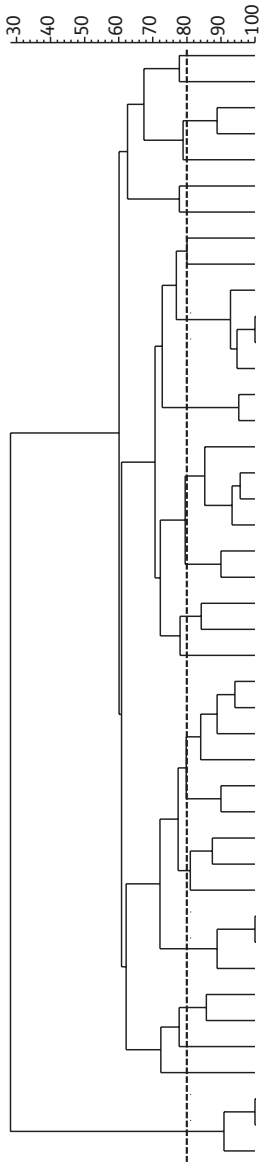
The 43 samples originated from animals admitted to 30 different clinics from 10 different cantons in Switzerland, indicating that MRCoNS are widespread and not related to a specific clinic with nosocomial infection problems (Figure 1). MRCoNS were isolated from different infection sites in cats ($n=16$), dogs ($n=20$) and horses ($n=7$) (Tables 1 and 2). Infected sites consisted of the skin ($n=10$), urinary tracts ($n=9$), ears ($n=4$), respiratory tracts ($n=5$), joints ($n=4$), eyes ($n=1$) and abscesses/fistulas ($n=10$) (Tables 1 and 2). The MRCoNS were identified as *S. epidermidis* (Table 1) and *S. haemolyticus*, *S. hominis*, *S. warneri*, *S. capitis* and *S. cohnii* (Table 2). Three samples contained an additional pathogen, i.e. *Staphylococcus schleiferi* together with *S. haemolyticus* KM785-09, *Staphylococcus pseudintermedius* together with *S. epidermidis* KM1077-09 and a mix of anaerobic bacteria together with *S. epidermidis* IMD1522-11.

PFGE revealed a large heterogeneity between the MRCoNS of the same species. Eight different PFGE clonal lineages were identified among the 17 *S. haemolyticus* isolates, 11 among the 20 *S. epidermidis* isolates and 1 among the 3 *S. hominis* isolates (Figure 1). The *S. epidermidis* strains showed distinct MLST patterns and belonged to 14 different sequence types (STs), representing 11 clonal complexes (CC), namely CC2 [ST2 ($n=4$), ST446 ($n=1$)], CC5 [ST5 ($n=3$), ST445 ($n=1$)], CC22 [ST22 ($n=3$)], CC59 [ST59 ($n=1$), ST81 ($n=1$)], CC17 [ST69 ($n=1$), CC89 [ST89 ($n=1$)], CC130 [ST450 ($n=1$)], CC166 [ST449 ($n=1$)], CC286 [ST286 ($n=1$)], CC451 [ST451 ($n=1$)] and CC448 [ST448 ($n=1$)]. ST445, ST446, ST448, ST449, ST450 and ST451 were newly described STs [Figure 1 and Figure S1 (available as Supplementary data at *JAC* Online)]. All but one of the isolates belonging to the predominant CC2 ($n=5$), CC5 ($n=4$), CC22 ($n=2$) and CC59 ($n=2$) clustered into four distinct PFGE branches (Figure 1). However, different PFGE patterns could still be observed within these groups, indicating a larger diversity between strains of the same CC (Figure 1).

Ten of 20 *S. epidermidis* isolates harboured the biofilm formation operon *ica*, including all the CC2 (ST2, ST446) ($n=5$) and ST22 ($n=2$) isolates as well as the ST69, ST448 and ST449 isolates. The *icaA* gene was also detected in one *S. hominis* isolate. ACMEs were only detected in *S. epidermidis*. Eight *S. epidermidis* isolates carried a type I ACME (*arcA* + /*opp3AB* +) and three carried a type II ACME (*arcA* + /*opp3AB*−). ACMEs were found in all ST5 and ST22 isolates, but were also present in one ST2 isolate and in the ST59, ST69, ST446, ST449 and ST450 isolates (Figure 1). An SCC*mec* element could only be typed for 17 isolates. SCC*mec* IV was detected in one *S. capitis*, one *S. warneri* and eight *S. epidermidis* isolates. In *S. epidermidis*, SCC*mec* IV was associated with ACME type I in the ST5, ST69 and ST450 isolates and with ACME type 2 in the ST59 isolate. SCC*mec* V was detected in seven *S. haemolyticus* isolates (Figure 1). The other 26 SCC*mec* elements could not be characterized as they lacked either known *ccr* genes or a known *mecA* class structure or both (Figure 1).

Distribution of MRCoNS isolates in veterinary practices

Association of a specific clonal lineage with a clinic was only observed for two pairs of *S. haemolyticus* (IMD1761-11, IMD1768-11 and IMD1632-08, IMD1758-08) isolated from different



Strain	Species	ST	animal	clinic	group	SCC*mec*	ACME	biofilm
IMD1530-11	*S. warneri*		dog	ZH8	R	IV	negative	
IMD1771-11	*S. cohnii*		dog	BL1	S	NT	negative	
IMD1528-11	*S. epidermidis*	ST448	cat	BL4	CC448	NT	negative	*icaA*
IMD1776-11	*S. epidermidis*	ST286	cat	ZH7	CC286	NT	negative	
IMD1764-11	*S. epidermidis*	ST449	dog	TI1	CC166	NT(C4B)	type II	*icaA*
KM1077-09	*S. epidermidis*	ST2	dog	VD3	CC2	NT(4A)	type I	*icaA*
KM825-09	*S. epidermidis*	ST451	horse	BL2	CC451	IV	negative	
IMD1265-11	*S. epidermidis*	ST69	horse	BL2	CC17	IV	type I	*icaA*
KM794-06	*S. epidermidis*	ST89	horse	BE2	CC89	NT	negative	
IMD1778-11	*S. epidermidis*	ST5	dog	BL1	CC5	IV	type I	
IMD1274-11	*S. epidermidis*	ST5	cat	ZH1	CC5	IV	type I	
IMD1766-11	*S. epidermidis*	ST5	dog	ZH5	CC5	IV	type I	
IMD1269-11	*S. epidermidis*	ST445	cat	ZH6	CC5	NT	negative	
KM1527-07	*S. epidermidis*	ST22	cat	VD2	CC22	NT(4A)	type II	*icaA*
KM505-09	*S. epidermidis*	ST22	cat	GE1	CC22	NT(4A)	type I	*icaA*
IMD1270-11	*S. epidermidis*	ST2	cat	ZH4	CC2	NT(4B)	negative	*icaA*
IMD1765-11	*S. epidermidis*	ST2	dog	ZH3	CC2	NT	negative	*icaA*
KM92-09	*S. epidermidis*	ST2	dog	VD1	CC2	NT(CA)	negative	*icaA*
CSNO38	*S. epidermidis*	ST446	horse	BE2	CC2	NT	type I	*icaA*
IMD1763-11	*S. epidermidis*	ST81	cat	BL3	CC59	IV	negative	
KM827-09	*S. epidermidis*	ST59	cat	GR1	CC59	IV	type II	
KM1385-1972	*S. epidermidis*	ST450	dog	BE1	CC130	IV	type I	
IMD1532-11	*S. haemolyticus*		dog	TI1	L	V	negative	
IMD1521-11	*S. haemolyticus*		cat	VD5	M	NT	negative	
KM1632-08	*S. haemolyticus*		cat	BE1	C	NT	negative	
KM1758-08	*S. haemolyticus*		cat	BE1	C	NT	negative	
KM1183-09	*S. haemolyticus*		horse	BE2	D	V	negative	
IMD1519-11	*S. haemolyticus*		cat	AG1	E	V	negative	
IMD1266-11	*S. haemolyticus*		dog	BE4	H	NT(CA)	negative	
IMD1397-11	*S. haemolyticus*		dog	U1	I	NT	negative	
IMD1775-11	*S. haemolyticus*		dog	SH1	F	V	negative	
KM1230-09	*S. haemolyticus*		horse	BE2	G	V	negative	
IMD1517-11	*S. haemolyticus*		cat	SH2	J	V	negative	
IMD1761-11	*S. haemolyticus*		dog	ZH1	A	NT	negative	
IMD1768-11	*S. haemolyticus*		dog	ZH1	A	NT	negative	
IMD1272-11	*S. haemolyticus*		cat	AG2	K	NT	negative	
IMD1277-11	*S. haemolyticus*		cat	GE2	N	V	negative	
KM785-09	*S. haemolyticus*		dog	BE1	O	NT	negative	
KM827-07	*S. haemolyticus*		horse	BE2	P	NT	negative	
KM1385-1970	*S. capitis*		dog	BE1	Q	IV	negative	
IMD1515-11	*S. hominis*		dog	AI1	T	NT	negative	
IMD1516-11	*S. hominis*		dog	ZH9	T	NT	negative	*icaA*
IMD1762-11	*S. hominis*		dog	ZH2	T	NT	negative	

Figure 1. Phylogenetic tree constructed from the PFGE pattern of methicillin-resistant *S. epidermidis, S. haemolyticus, S. hominis, S. capitis and S. warneri*. The tree was generated by UPGMA using Bionumerics 6.6 (Applied Maths, Kortrjk, Belgium) and comparison settings (Dice, optimization 1.5%, position tolerance 1.5%) as recommended by PulseNet International (www.pulsenetinternational.org). The broken line indicates the cut-off value of ≥79%, determining clonality between the isolates according to Miragaia *et al*.[47] Capital letters indicate the cantons and the numbers indicate the different clinics. AG, Argovia; AI, Appenzell Inner Rhoden; BE, Bern; BL, Basel-Land; GE, Geneva; GR, Grisons; SH, Schaffhausen; TI, Ticino; VD, Vaud; ZH, Zurich; U, unknown.

animals in two clinics. Each pair showed similar PFGE profiles (A and C) and contained a non-typeable SCC*mec* element (Figure 1). However, they exhibited different antibiotic resistance profiles (Table 2). Otherwise, MRCoNS isolated from animals admitted to the same clinic were genetically distant. On the other hand, genetically related MRCoNS were isolated from different animals in different clinics (Figure 1). These isolates also displayed different antibiotic resistance profiles (Tables 1 and 2).

Antibiotic resistance profile

All the MRCoNS isolates were resistant to β-lactam antibiotics and contained the *mecA* gene. None of them was resistant to linezolid, quinupristin/dalfopristin, rifampicin or vancomycin. Nonetheless, the isolates were also resistant to gentamicin/kanamycin owing to the bifunctional acetyltransferase/phosphotransferase gene *aac(6′)-Ie–aph(2′)-Ia* ($n=33$), kanamycin [*aph(3′)-III* ($n=17$)], macrolides and/or lincosamides [*erm*(C) ($n=22$), *erm*(A) ($n=1$), *msr* ($n=12$) and *lnu*(A) ($n=4$], tetracycline [*tet*(K) ($n=22$)], trimethoprim [*dfr*(A) ($n=7$) and *dfr*(G) ($n=10$)], streptomycin [*str* ($n=5$) and *ant(6)-Ia* ($n=15$)], streptothricin [*sat4* ($n=15$)], chloramphenicol [cat_{pC221} ($n=5$) and cat_{pC223} ($n=3$)], tiamulin [(*vga*(A) ($n=4$)], mupirocin [*mupR* ($n=2$)], fusidic acid ($n=13$), sulfamethoxazole ($n=34$) and fluoroquinolones ($n=30$) (Tables 1 and 2). Resistance mechanisms

for fusidic acid and sulfamethoxazole were not investigated. Fluoroquinolone resistance was attributed to mutations in topoisomerase II GyrA ($n=30$) and topoisomerase IV GrlA ($n=18$) (Tables 1 and 2). Mutations that cause amino acid substitutions in topoisomerases II and IV were found in ciprofloxacin-resistant *S. epidermidis*, *S. haemolyticus* and *S. hominis* at nucleotide position 251 [$n=30$; Ser84Leu ($n=14$), Ser84Phe ($n=13$), Ser84Tyr ($n=3$)] in *gyrA* and at positions 239 [$n=8$; Ser80Tyr ($n=4$), Ser80Phe ($n=4$)] and 250 [$n=10$; Asp84Tyr ($n=7$), Gly84Tyr ($n=3$)] in *grlA*. An amino acid substitution in GrlB (Glu473Lys) was also present in two *S. epidermidis* isolates (KM505-09 and KM1527-07) and two *S. haemolyticus* isolates (IMD1277-11 and IMD1532-11). This mutation was not considered as being responsible for fluoroquinolone resistance in CoNS, as a mutation at the same location has been shown not to confer resistance to fluoroquinolones in *Staphylococcus aureus*.[38] The resistance mechanism could not be explained for one strain with resistance to gentamicin and kanamycin, for one strain with resistance to clindamycin and for two strains with decreased susceptibility to tiamulin (MIC >4 mg/L), suggesting new mechanisms of resistance.

Clinical data of infected animals

Clinical data were obtained for 27 animals (11 dogs, 11 cats and 5 horses) admitted to 19 different clinics (Table 3). Twenty-four animals had a history of antibiotic treatment, and 20 of them underwent antimicrobial treatment more than twice with up to 14 courses. The most commonly used antibiotics in dogs and cats prior to the identification of the staphylococcal species were amoxicillin/clavulanic acid, cephalosporins and fluoroquinolones. In 20 dogs and cats treated, amoxicillin was given 15 times, fluoroquinolones 8 times, cephalosporins 6 times, and both a β-lactam and a fluoroquinolone antibiotic were given 7 times. Antibiotics such as gentamicin, chloramphenicol, tetracycline and clindamycin were also used in pets, but less frequently. In horses, cefquinome, fluoroquinolones and the combination penicillin/gentamicin were the most commonly administered antibiotics. The MRCoNS infections were then treated after consultation of an antibiogram, most frequently using fluoroquinolones or amoxicillin/clavulanic acid followed by tetracycline, clindamycin, chloramphenicol, cefalexin, the combination sulphonamides/trimethoprim, rifampicin and the aminoglycosides gentamicin, framycetin and neomycin. Most of the animals ($n=14$) recovered after antibiotic treatment: six had a relapse, two recovered without any antibiotic therapy, one recovered after amputation of the infected lower extremity, one was still under treatment at the time of writing, two were not further treated and euthanized and one died of unknown cause prior to therapy. In seven cases, antibiotics were used even in the presence of resistance, leading to relapse in two cases when cefalexin and gentamicin were used for the treatment of an abscess and skin infection, respectively. The other five animals recovered after antimicrobial treatment with amoxicillin/clavulanic acid ($n=3$), clindamycin ($n=1$) or framycetin ($n=1$), despite the presence of *mecA*, *erm*(C) or *aac(6′)-Ie–aph(2′)-Ia* in the respective MRCoNS (Table 3). For these animals, MRCoNS were likely not the primary cause of the infection (three urinary tract and two ear infections), although MRCoNS appeared alone in the culture. No significant difference was observed in the outcome of the disease between animals treated with an antibiotic incompatible and an antibiotic compatible with the resistance profile of the MRCoNS.

Discussion

MRCoNS are associated with serious infections in animals and have become a challenge to therapy. The CoNS species identified in this study were the same as the ones causing nosocomial infections in humans, with *S. epidermidis* and *S. haemolyticus* being the most prevalent in animals and humans.[7] Similar to the case with human infections,[11,13] *S. hominis*, *S. warneri*, *S. cohnii* and *S. capitis* were only occasionally isolated from animal infection sites. The population analysis by PFGE showed that the majority of the isolates are genetically diverse. Three clonal lineages sharing similar PFGE profiles appeared to be predominant among *S. epidermidis* and were found to belong to CC2, CC5 and CC22. However, isolates of CC2 and CC22 contained divergent SCC*mec* elements and ACMEs, while CC5 isolates almost exclusively contained SCC*mec* IV and ACME type I. Additionally, these clonally related isolates displayed different resistance profiles, emphasizing the ability of CoNS to acquire antibiotic resistance genes. The presence of numerous PFGE and antibiotic resistance profiles is a well-described phenomenon for *S. epidermidis* ST2, which is the most widely disseminated human healthcare-associated sequence type worldwide.[39–42]

A study using 217 *S. epidermidis* isolates from humans from 17 countries detected 30.9% of the isolates as ST2.[42] The successful spread of ST2 in the hospital environment has been suggested to be associated with its ability to generate novel phenotypic and genotypic variants by recombination and acquisition of new elements, such as the biofilm-formation *ica* operon, ACMEs and antibiotic resistance genes.[14,15,18,41] In our study, all isolates of CC2 and CC22, which is a subcluster of CC2 (Figure S1, available as Supplementary data at *JAC* Online), contained the biofilm formation operon *ica*. On the other hand, the *ica* operon was absent in isolates of CC5, which was also predominant in infection sites of animals; they contained ACMEs instead. Of note, CC22 contained both *ica* and ACMEs. The presence of the biofilm formation operon *ica* and ACMEs almost exclusively in *S. epidermidis* of the predominant clonal lineages CC2, CC5 and CC22 may have contributed to the establishment of these strains in the animal environment. In addition to the predominant STs, the animals were also infected with other *S. epidermidis* strains, which has also been reported in human infections, such as ST35, ST59, ST81, ST69, ST89 and ST286 (Figure S1, available as Supplementary data at *JAC* Online),[40–42] and with *S. haemolyticus*. The absence of MLST methods for *S. haemolyticus* prevented us from determining whether specific STs would also be predominant within this species. However, the different PFGE and antibiotic resistance profiles support the hypothesis that MRCoNS associated with infections in animals are very heterogeneous, unlike methicillin-resistant *S. pseudintermedius* (MRSP), which spread as specific clones.[43] Nevertheless, MRSP and MRCoNS are resistant to the same classes of drugs and contain similar antibiotic resistance genes. Similar to MRSP, more than two-thirds of the MRCoNS exhibit resistance to fluoroquinolones, macrolides, lincosamides and aminoglycosides, in addition to resistance to β-lactams, suggesting that they have been selected

through the frequent use of antibiotics. These classes of drugs, especially the β-lactams and fluoroquinolones, were also the most commonly used drugs in veterinary practices (Table 3). These two classes of drugs have been shown to represent a significant risk factor for the selection of methicillin-resistant *S. aureus*[44] and similar effects are to be expected for MRCoNS. Many animals were given more than one antibiotic course, with some animals receiving 5 and up to 14 courses of an antibiotic before the MRCoNS infection was diagnosed. The series of empirical antimicrobial treatments may have contributed to the selection of the MRCoNS in the infection sites. Additionally, the primary cause of the infection may have been overlooked and not directly related to the presence of a MRCoNS. Indeed, two animals recovered without antibiotic treatment and five recovered despite the presence of a resistance mechanism against the antibiotic used for treatment. Nevertheless, in the majority of the cases the staphylococcal infections could be treated with an antibiotic chosen after consultation of an antibiogram. All these criteria highlight the importance of correct diagnosis and antibiograms.

Multidrug-resistant CoNS represent a new challenge for therapy in veterinary medicine. The infections are caused by genetically distant strains, indicating many possible non-hospital-related reservoirs, such as animals themselves, animal owners and people working with animals that have been shown to harbour and possibly exchange MRCoNS.[22,45,46] The presence of clones similar to those causing infections in humans highlights the importance of careful surveillance of bacterial infection diseases, the need to implement infection control programmes and the prudent use of antibiotics in veterinary settings.

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Transparency declarations

None to declare.

Supplementary data

Table S1 and Figure S1 are available at *JAC* Online (http://jac.oxfordjournals.org/).

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